The limits to artificial selection for body weight in the mouse

IV. SOURCES OF NEW GENETIC VARIANCE—IRRADIATION AND OUTCROSSING

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1. INTRODUCTION

In an earlier paper (Roberts, 1966*b*), it was established that a line of mice selected for high 6-week weight, after it had reached its limit to selection, contained no residual additive genetic variance in the trait. There was no regression of the weight of the offspring on that of the sire, and reversed selection failed to bring about any decrease in body weight. Though difficult to prove conclusively, it was suggested that most loci contributing to variance in body weight had been fixed in the population. This paper reports two attempts to introduce new genetic variance into the line, that would lead to a renewed response to selection for high 6-week weight. The first method attempted was irradiation; the second method was to outcross the selected line to a random-bred unselected population.

The induction of new genetic variance in quantitative characters by means of irradiation, more specifically X-irradiation, has been the subject of much comment. There are several references to the successful utilization of the method to improve varieties of commercial plants, though these successes may have required relatively high doses of irradiation—of the order of 100,000 r. Animal populations cannot be exposed to such high doses, and the results of similar studies on them are correspondingly less striking. Some early attempts to accelerate the advance under selection (for sternopleural bristles in *Drosophila*) by means of irradiation gave largely negative results (Serebrovsky, 1935; Rokizky, 1936). But a completely different outcome was reported by Scossiroli (1954). By alternating irradiation (3000 r. per generation) and selection, he increased spectacularly the number of sternopleural bristles in lines of *Drosophila* that had reached their limit to previous selection for a high score, although his low lines showed little further response under the same procedure. A similar experiment (Scossiroli & Scossiroli, 1959) using isogenic material gave essentially the same result. Clayton & Robertson (1955, 1964) employed a similar approach, but reported only modest gains, and were cautious about the general usefulness of the method, at least for mammals. Using 1800 r. per generation, they obtained small but consistent responses to selection for

sternital and sternopleural bristles from inbred lines of *Drosophila*. They calculated that 500,000 r. would be required to raise the genetic variance of an inbred line to the level of a standard outbred population. When the same dose of 1800 r. per generation was applied to seven selected lines that had reached their limits, the additional responses (above those of the non-irradiated controls) were likewise uniformly small, though significantly greater after irradiation in three of the seven lines.

Abplanalp, Lowry, Lerner & Dempster (1964) describe an attempt to exploit X-ray-induced mutations in subsequent selection for egg number in poultry. A total dose of 8000 r. was applied to chicken sperm over seven generations. Selection for a further six generations failed to show any improvement of the irradiated lines over their non-irradiated controls, and the authors conclude that the dose of 8000 r. did not induce sufficient new genetic variance to help selection for high egg number in chickens.

Though the evidence from the literature is inconsistent, it indicated that irradiation should be attempted as a method of inducing genetic variance in the line of mice that had ceased to respond to selection on account of the fixation of loci affecting body weight. In parallel with this study, a second method was explored. The large line was outcrossed to an unselected population to test whether any alleles were available in that population which were more favourable than the ones fixed in the large line. Some encouraging results from this method have been obtained recently by Robertson & Osman (private communication). They outcrossed to the base population a line of *Drosophila* that had reached its limit for low number of sternopleural bristles, selecting from the outcross to see how soon the original limit might be transcended, and by how much. Though, on average, their extra gains were small, some of their replicates surpassed the original limit by a substantial margin.

The *Drosophila* experiments suggest that both methods of introducing new genetic variance—by irradiation and by outcrossing—may result in some advance under further selection to a point beyond the previous limit. But the results are variable, and there appears to be little information about the general utility of the two methods for any mammal. The experiments described below were designed to explore their potential, using a line of mice that had reached its limit, and to formulate more clearly any problems that may have to be overcome when the methods are applied to a mammalian population.

2. THE FIRST EXPERIMENT—IRRADIATION

(i) *Materials and Methods*

The line of mice employed for this study was the *CL* line, described by Roberts (1966*b*). In generation 35, fifteen pairs of sibs were selected on the basis of their 6-week weights. The heavier and lighter member of each pair were assigned alternately into two matching groups. One group of fifteen males was then employed for continuation of the *CL* line while the other fifteen males were irradiated. After

ensuring that both testes were in the scrotum, the body was shielded except for the scrotal region, and a dose of 600 r. was applied to each male.

This dose produces many chromosomal aberrations in irradiated spermatids and later stages in the mouse. The males remain fertile for a short time and then they enter a sterile period. Six weeks or so after the irradiation, the males recover fertility, the mature sperm having been in the spermatogonial stage when irradiated. The gametes no longer contain chromosomal aberrations but they are expected to carry mutations at a frequency perhaps fifteen times higher than that in non-irradiated males. Female mice can hardly be irradiated at all without inducing complete sterility; even a dose as low as 50 r. destroys all early oocytes. The litera-ture on the effects of irradiation on mammalian (especially mouse) germ cells is voluminous. A useful summary of the subject, as it governed the choice of pro-cedures for this experiment, is provided by Russell, Russell & Oakberg (1958).

In view of the sterile period following irradiation, the fifteen males were not used for 3 months. One died during this time, but the surviving fourteen were then mated to females drawn from the succeeding (36th)generation of *CL*. These females were drawn in a manner identical to that described for the irradiated males. The mating was at random except for the avoidance of close relatives. A replicate of the *CL* line was therefore produced, the only difference between it and the parent line being the irradiation of the males with 600 r. This was the zero generation for further selection in an attempt to exploit any favourable mutations induced by the irradiation.

The mechanics of the selection programme to be followed will depend on whether the new mutations to be exploited (if favourable) are recessive or not. If they are dominant or semi-dominant, there is no problem; they will contribute to the variance in body weight of the progeny of the zero generation and will be selected in the normal course of events. But if they are recessive, steps must be taken to make them homozygous before they can be selected, and difficulties arise. Each mutation will presumably be a unique event and appear in only one gamete of the irradiated males. They will therefore be borne by single animals, and in the heterozygous state, in the first generation. However, after these heterozygotes have bred, half of their offspring (i.e. generation 2) will be expected to carry the particular mutation in which we may be interested. If these offspring are now sib mated, a quarter of all matings ought to be between animals heterozygous for the same original mutant; bearing in mind that in the grand parental generation there were two irradiated males, a quarter of the matings also could be between animals heterozygous for a mutant from the second male, should that male as well have passed on an autosomal mutant to one of its progeny. Uncertainties about the number of loci involved, and the number of mutations an irradiated gamete may be expected to carry, rule out any probability statements about the frequency of homozygotes to be expected following sib mating, but subjectively, the procedure was thought to be worth the attempt.

Hence, at generation 2, the irradiated line was split. One subline was designated *ID* (*I* for 'irradiation' and *D* for 'dominants'); this line aimed to exploit dominant

and semi-dominant mutations at loci affecting body weight, and was selected and mated in the same way as the parent CL line. The other subline was designated IR (R for 'recessives') and the generation consisted of matings between sibs, for the reasons given above. The progeny of the sib matings were then selected, hopefully to pick out any desired recessive homozygotes, and then mated according to the usual scheme of avoiding close relatives. The sib mating, with selection, was repeated twice, separated by a generation of selection and mating without inbreeding. Thereafter the IR line was treated in the same way as ID.

Both lines, and also the parent CL line were run on fifteen pair matings. Within-family selection was practised throughout, to avoid complications due to maternal effects in the interpretation of the results.

The irradiation was applied once only, in the beginning. In retrospect, this was perhaps a mistake, but at the time, a single acute dose was deemed sufficient to test the general utility of the method. A purely operational point of view was taken—'Does 600 r. give us anything worth while?'. With the power of hindsight, a better question might have been—'What total dose is required to give us anything at all?'

(ii) *Results and Discussion*

The main results from the irradiation experiment are presented in Fig. 1. This shows the mean 6-week weights of the two irradiated lines compared to those of the parent CL line (the dotted parts of the IR graph refer to the sib-matings, mentioned above). The interpretation of the results is complicated slightly by an increase in weight in the CL line at the 44th generation. This increase was discussed fully by Roberts (1966b) and attributed to a genetic change in the line, most probably a rare recombinational event, though some increase due to environmental causes could not be completely discounted. Figure 1 now confirms that there was no marked increase from a general environmental influence at the time; the IR and ID lines failed to show any parallel increase, the generations in vertical alignment in the figure being roughly contemporaneous in all cases. We should therefore judge whether there has been any additional response to selection in the IR and ID lines, following the irradiation, by comparing them with the old level of CL, i.e. generation 43 and earlier. The limit prior to this point was established as 32 g., whereas subsequently it rose to 35 g. (Roberts, 1966b).

Nine generations after the irradiation, there was no evidence that selection in the ID and IR lines had increased weights at all over the original level of 32 g. Then both lines showed some increase. The two irradiated lines by this time were running so obviously in parallel that the ID line was discontinued after generation 12. The remaining line, IR, settled down to a level on average about a gramme or so higher than the original limit in the CL line.

The question then arises whether this rather small increase was attributable to a response following the irradiation. Quite obviously, it might have been, but it is hard to say for certain. Changes in the weights of selected lines of mice at their

limits are common, as seen from an earlier paper in this series (Roberts, 1966a). In any other circumstances, a shift of this magnitude in mean weight would attract little attention, for environmental trends in long-term experiments may always be suspected. But if we were prepared to dismiss this possibility, the following points could be listed in favour of the possible effects of the irradiation:
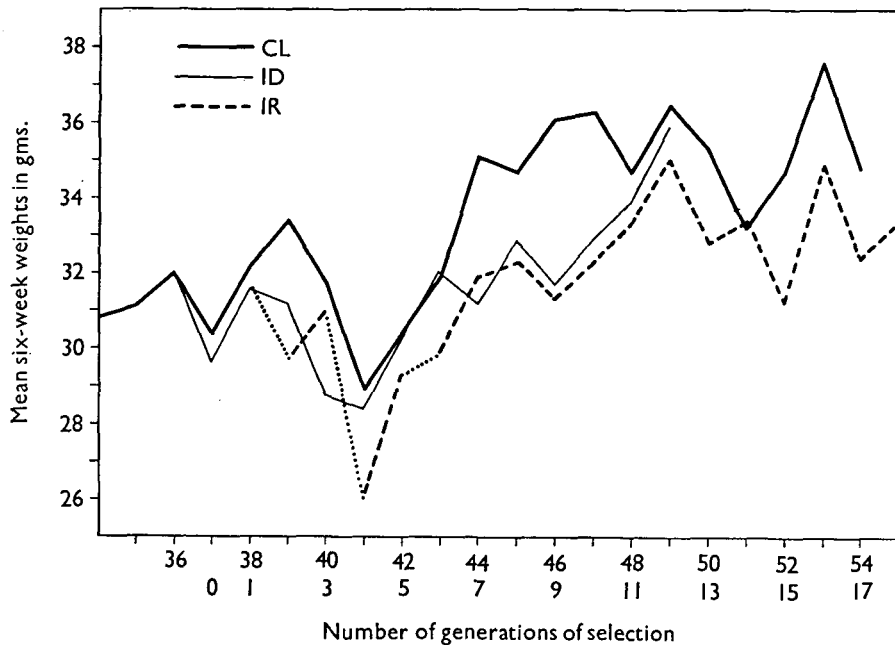

Fig. 1. Responses to selection for body weight following irradiation in *ID* and *IR* lines. Non-irradiated parent line (*CL*) shown for comparison. The dotted parts of *IR* line correspond to generations of sib-mating (see text).

(1) There was a slight increase in absolute terms, as mentioned.

(2) The postulated recombinational event that increased the weight of *CL* must be so rare that it cannot be invoked a second time; nor should any other rare recombination occur synchronously in both irradiated lines.

(3) There was some suspicion of an increase in variance within sexes within litters—the variance upon which the selection acted—following the irradiation. This increase over the *CL* variance, however, was by no means consistently a feature of the irradiated lines over succeeding generations.

(4) In one of the irradiated lines (*IR*) the regression of offspring weight on that of the sire became significantly positive ($+0.133 \pm 0.059$). This contrasts sharply with the position in the *CL* line and some of its other derivatives (Roberts, 1966b). Counterbalancing this argument, the same regression in *ID* was much less than its standard error, to which we may add the fact that in the remainder of the material for this series of papers, a sufficient number of

insignificant regressions of this type have been calculated that an apparently significant one, by chance alone, should be expected.

(5) Possibly the strongest argument in favour of an effect of irradiation is that, if there was any effect, the increase in weight found is very much what would be expected, if Clayton & Robertson's (1964) *Drosophila* bristles provide any lead.

To summarize, the main conclusion is that a gonad dose of 600 r. to male mice, from a selected line at its limit, did not contribute to any substantial advance under further selection. But there may have been *some* advance. If an experiment were planned to investigate this possibility further, the following recommendations could now be made:

(1) A single dose of 600 r. is too small, in the light of general experience with other organisms. Of particular interest in this context is Russell's (1962) finding that X-rays delivered in two fractions of 500 r., separated by 24 hours, gives a mutation rate of five times that observed for a single 1000 r. dose given to male mice.

(2) As success, if any, may be sporadic and unpredictable, a proper experiment on irradiation effects should incorporate several replicates.

(3) The control lines should also be replicated, to safeguard against fortuitous shifts of the kind found in the *CL* line.

(4) To allow clear patterns to emerge, the considerable facilities that all this involves may have to be committed to the project for at least twenty generations.

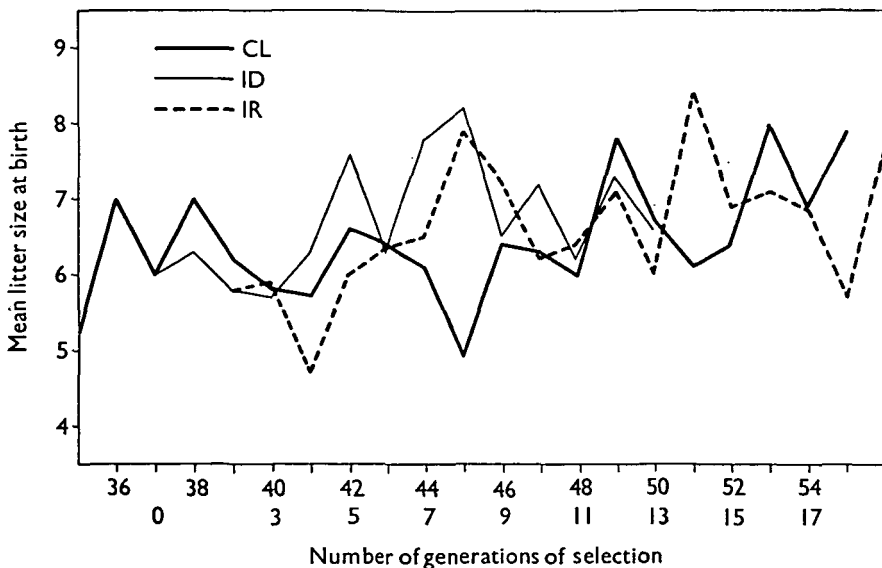As a postscript, in view of the general interest in the effects of irradiation on

Fig. 2. Effects of irradiation on litter size in *ID* and *IR* lines. Non-irradiated parent line (*CL*) shown for comparison.

fertility, the mean numbers of live young in the first litters of the three lines are shown in Fig. 2. Litter size, while it may not have increased much following the irradiation, certainly did not show the expected decline.

3. THE SECOND EXPERIMENT—OUTCROSSING

(i) *Materials and Methods*

It was explained earlier that the purpose here was to comb a random-bred population for any alleles more favourable to high weight than those fixed in the *CL* line. A potentially useful source of such alleles was locally available in Dr D. S. Falconer's *Q* strain, whose origin and structure is described in *Mouse News Letter* (No. 25, p. 29). The strain was constructed from a broad base, though one which had considerable overlap with the *CL* line.

The procedure for drawing two matching samples from the *CL* line was described in the previous section, when choosing fifteen males to be irradiated. In an identical manner, fifteen females were drawn from the same (35th) generation of *CL*. These were mated to fifteen males from the *Q* strain to form the zero generation of the *CQ* line, from which to select for an increase in 6-week weight. Two questions were asked of the selection programme:

(1) How long would it take to restore the mean weight to the level of *CL*? There was no reason why this level should not be regained, since genes that had been fixed in *CL* were at a frequency of at least 50% in the *CQ* population. If alleles from *CL* were superior at all loci to those available in *Q*, it would merely be a question of making them homozygous again to restore the level of the *CL* body weights.

(2) Could the limit reached in *CL* be transcended, and if so, by how much? This is equivalent to asking whether any of the *Q* alleles were superior to those fixed in *CL*.

Depending on the effect of the infusion of genes from the *Q* strain, it was anticipated that some selection would be required to nullify the effects of the outcross on body weight. It might therefore be advantageous to start the new selection from a higher level. To test this, the matching procedure was employed again to divide females of the zero generation of *CQ* into two groups. One group was mated to males selected from the same generation to continue the *CQ* line, while the other group of females was backcrossed to males of the 36th generation of *CL*, which were again drawn alternately from selected sib pairs. This second set of matings was designated the zero generation of the *CQB* line, which from that time was treated exactly the same as the *CQ* line.

It was expected that *CQB* might regain the original limit of *CL* sooner than *CQ*, but that this might involve some sacrifice of the total advance. The reason for this would be that any favourable alleles from *Q* that were at a low frequency might be lost during the backcrossing, by sampling. But if favourable alleles were at a high

frequency in the Q stock, the backcrossing should not affect greatly their availability for further selection.

The approach taken at the start of these studies was quite empirical, and the results are presented here as such. However, the implications of the procedure and its theoretical basis will be described in more depth in a forthcoming paper by Robertson & Osman (private communication), who carried out a similar but more extensive study on *Drosophila*.

As in other lines described in this series of papers, CQ and CQB were both run on fifteen pair matings per generation, and the within-family method of selection was employed throughout.

(ii) *Results and Discussion*

The mean 6-week weight of the Q strain when it was crossed to CL was about 22 g. The mean weight of the F_1 was above the mid-parental value, as expected. This is because directional dominance is towards large size, and also because of the maternal effect deriving from the use of the large CL mice as the dams during the crossing.

The progress of the CQ line from zero generation is shown in Fig. 3. After six generations of selection, the weights were actually lower than at the starting point. There was then a steady response for perhaps ten generations, after which weights fluctuated erratically around a mean of 36 g. or so.

The CQB line shows a very similar pattern of response. On backcrossing (using CL males on CQ females), the weights were actually lower than those obtained in the CQ line (CQ females by CQ males). This was probably an accident of sampling, but over the succeeding generations there is little evidence that the weights had been increased by the backcross over the level of the outcross. Again, as in CQ, the CQB line showed no advance under further selection for six generations. This was followed by a sharp response, and by the 12th generation, it appeared as if CQB was going to exceed the level attained by the CQ line. But eventually both lines settled down to much the same weight, at around 36 g.

The main interest in this study concerns the final limit of the CQ and CQB lines compared to the initial limit of 32 g. in the CL line. (The complication of a later rise in CL is disregarded for reasons given earlier.) There is no doubt that genes have been extracted from the Q strain that enable the limit of the CL line to be surpassed. This means that less favourable alleles had been fixed at some loci in the CL line during the earlier selection.

However, the Q strain has proved less useful as a source of new variance in the CL line than did three selected large lines, in a study described by Roberts (1967). There the gain ultimately attained over the CL level was fully twice that reported here.

A point of considerable practical importance is the length of time required after the outcross to recover the weight of the selected line. Again taking 32 g. as the original level of the CL line, this weight was reached by the 9th generation in both CQ and CQB. As CQB had required an extra generation for the backcross, it is

therefore marginally inferior on this score to the *CQ* line that was selected straight away from the outcross. The idea that it would be advantageous to start from a higher level, and recover the lost weight sooner, was thus not sustained in practice.

The lag of six generations after crossing before any response to selection was observed, is an exact repeat of the experience from selection following the crossing of selected strains (Roberts, 1967). The results from that study were interpreted in terms of linkage, and without repeating any of the argument, the same explanation is equally satisfactory here.

The close similarity between the *CQ* and *CQB* responses is quite striking. Generation *n* of *CQB* was always roughly contemporaneous with generation $(n+1)$ of *CQ*. This dispels the possibility that environmental trends affected the two lines synchronously. For instance, when *CQ* began to respond, *CQB* adopted the same
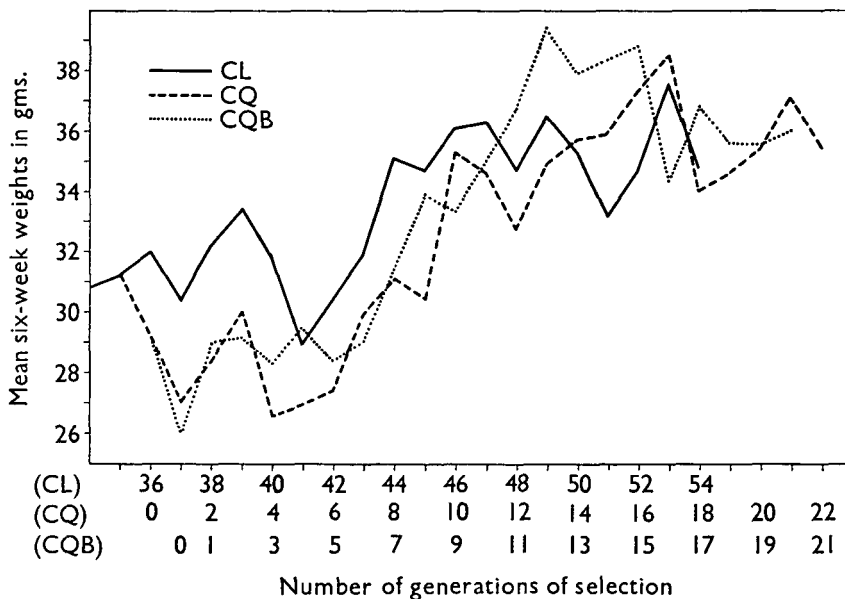

Fig. 3. Selection for body weight from cross between a line at its limit (*CL*) to a random-bred strain. *CQ*-selection from F_1. *CQB*-selection from backcross to *CL*.

pattern 3 months later. The similarity suggests that the *CQB* line was not adversely affected by the fact that new genes from *Q* had their frequencies halved by the backcrossing. This must mean that favourable alleles from *Q* were not at a low frequency in that strain; had they been rare, it would have taken a little while longer in *CQB* to increase their frequency, before their effects on the mean weight became noticeable.

Outcrossing, as a method of breaking through the limit, therefore seems to have been moderately successful. This encourages the thought that other outcrosses to unrelated populations might bring about further gains. This idea, of course, is not

new. Falconer (1960), discussing the relatively small divergences generated by laboratory selection experiments, compared to differences between breeds of livestock, wrote: 'The reason for the disappointing results of experimental selection . . . is that experiments are carried out with closed populations of not very large size. The limits are set by the gene content of the foundation individuals. . . . The breeder of domestic animals, in contrast, by intermittent crossing casts his net far wider in the search for genes favourable to his purposes.'

4. GENERAL CONCLUSIONS AND IMPLICATIONS

Irradiation, as a means of generating new genetic variance, does not inspire much hope in terms of the improvement of mammalian populations. It is not easy to apply in practice, and the expected gains, if any, are small. If the study reported here is typical of what may be found, special attempts to extract favourable recessive mutations do not lead to any increased gains. This statement, however, may not have general validity. It is probably true of characters like large size, where favourable genes tend to be dominant. But the desired expression of other characters may involve recessive genes, and the prospects of their recovery from irradiated material is not good. For these traits, especially, irradiation as a method seems to have little to commend in it.

Outcrossing to an unselected population seems more hopeful, but it may take eight to ten generations to recover the level of the original limit. For most farm livestock, this is a discouraging prospect, and one that could not be undertaken by individual breeders. The time scale for most domestic animals would be 10 to 20 years, before any increased return might be expected. Only then could any of the lost production in the interim begin to be recovered. Even on the basis of a national scheme, stock improvement by outcrossing to genetically inferior material seems feasible only for rapidly reproducing species, where the breeding project need not encroach heavily on the current production facilities.

The greatest improvement over the initial limits, found in the investigations reported in this series of papers, came from crosses between selected strains with further selection from the crosses (Roberts, 1967). Even this method, though highly successful in increasing body weight, was not very effective in reducing body weight. It may therefore not always work in the desired direction. Where it was effective, it became associated with fertility problems that demanded special attention. But its main advantage over the outcrossing method was that there was no regression from the level of the initial limit, at least not with crosses between large strains. If small body weight were desired (as, for instance, in egg-laying strains of poultry) the increase in weight on crossing may detract from the value of the method, even without the doubt about the effectiveness of further selection for small size.

Linkage seemed to impede progress under further selection in all material involving crosses. It was suggested in the previous paper that this problem may be common in crosses involving highly selected lines or strains, since the unfavourable

alleles likely to be fixed are those linked to others affecting the trait. Linkage may reduce somewhat the further gains that may be made, but its chief nuisance value is in impeding the initial rate of advance, if the experiments reported in this and previous papers are representative.

In summary, the limits to artificial selection, being a function of the gene content of the selected material, need not be insuperable barriers to further progress if a useful source of better genes can be tapped. But with slow-reproducing mammals, especially, any method employed to transcend the limit is likely to be time-consuming and costly. The problems are those of organization and finance; the genetic methods have been examined and, within their context, evaluated in this series of papers.

SUMMARY

1. Two methods are examined of introducing new genetic variance into a line of mice selected for high 6-week weight which, at its limit, displayed no additive genetic variance.

2. The first method—irradiation—gave largely negative results. Any further gain under selection that was achieved could not be clearly distinguished from a possible environmental trend.

3. The second method—outcrossing to an unselected strain and then selecting from the cross—resulted in a clear gain over the original limit, but nine generations were required even to recover the original limit.

4. Various methods of transcending selection limits are evaluated in terms of their application to livestock improvement.

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